ANALYSIS VOLATILE COMPOUND OF GAHARU OIL COMPOSITION VIA SOLID PHASE MICRO EXTRACTION (SPME)

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of the requirements for the award of the degree of
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I declare that this thesis entitled "**Analysis Volatile Compound of Gaharu Oil via Solid Phase Micro Extraction (SPME)**" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

Signature :……

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Date : 30 April 2009

Special Dedication to my family members,
my friends, my fellow colleague
and all faculty members

For all your care, support and believe in me.

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ABSTRACT

Gaharu (agarwood) is a fragrant wood that is usually derived from the diseased timber of the genus Aquilaria Thymelaeceae and often occurs as dark coloured patches or streaks in the tree. Due to its strong, unique scent and medicinal properties, gaharu oil is greatly valued as perfumery ingredient and incense. Gaharu may be classified into various grades; Grade A, B, C and D and they are often graded according to the physical properties, gaharu formation and its unique scent. The lower grades such as Grade C are often distilled to obtain gaharu oils. As part of an on-going research on the chemical profiling of some Malaysian gaharu oils and evaluation of their potential beneficial properties; gaharu oils obtained from different sources were analysed and compared by SPME and GCMS. Identification of the chemical components was based on comparison of the types of SPME fibers and chromatographic columns. The SPME device included a fused silica fiber coating partially cross-linked with 100μm Polydimethylsiloxane (PDMS), 75μm Carboxen/Polydimethylsiloxane (CAR/PDMS) and 65μm Polydimethylsiloxane /divinylbenzene (PDMS/DVB). The chromatographic column used were HP-5MS 5% Phenyl Methyl Siloxane and DB-WAX , 30 m x 250 μm i.d, film thickness 0.25 μm. Examination of the oils showed some variations and differences in terms of GCMS profiles, concentration and chemical components. Majority of the essential oil profiles were complex and made up of sesquiterpenoids and their oxygenated derivatives. However, common occurrences of chemical compounds such as benzaldehyde, 3-phenyl-butanone, alpha-guaiene and gamma- guaiene were detected.

ABSTRAK

Gaharu (agarwood) adalah sejenis kayu wangian yang biasanya didapati daripada spesis *Aquilaria Thymelaeceae* dan mempunyai corak serta jalur berwana hitam. Berdasarkan sifat-sifat kekuatan, keunikan dan perubatan, minyak gaharu biasanya digunakan sebagai bahan pewangi dan kemenyan. Gaharu boleh dikelaskan kepada beberapa gred iaitu gred A,B,C dan D yang mana boleh dibeza berdasarkan sifat-sifat fizikal, pembentukan dan keunikan gaharu. Gred C iaitu gred yg rendah biasanya digunakan untuk penyulingan minyak gaharu. Penyelidikan berdasarkan bahan profil kimia dan kepentingan sifat-sifat dalam penghasilan minyak gaharu di Malaysia telah dibuat dengan menggunakan *SPME* dan *GCMS*. Identiti setiap komponen kimia diperolehi daripada perbezaan jenis fiber *SPME* dan kolum kromatografik *GCMS*. Jenis-jenis fiber *SPME* adalah *100µm Polydimethylsiloxane (PDMS), 75µm Carboxen/Polydimethylsiloxane (CAR/PDMS) dan 65µm Polydimethylsiloxane /divinylbenzene (PDMS/DVB).* Manakala kolum kromatografik adalah seperti *HP-5MS 5% Phenyl Methyl Siloxane dan DB-WAX , 30 m x 250 µm i.d,* ketebalan filem *0.25 µm.* Penyelidikan terhadap minyak gaharu menunjukkan perbezaan dalam bentuk profil GCMS, kepekatan minyak dan komponen-komponen kimia. Kebanyakan komponen seperti *sesquiterpenoids* dan kompaun sampingan hasil pengoksidaan sangat susah didapati dalam penghasilan profil minyak wangi. Walau bagaimanapun, di dalam analisa ini komponen-komponen seperti *benzaldehyde, 3-phenyl-butanone, alpha-guaiene and gamma- guaiene* diperolehi.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Gaharu or agarwood is the resinous heartwood from aguilaria tree that is the occasional product of two to four general in the family Thymelaeaceae, with scientific name are Aquilaria agallocha, Aquilaria crassna and Aquilaria malaccensis Lam. This is the best unknown species. This resin is commonly named Aloeswood, Jinko, Jin Koh, Eagle wood, Oud or Ood ud. It's an evergreen tree native to Asia like northem India, Laos, Cambodia, Malaysia, Indonesia and Vietnam.

Gaharu has been used as medicine, perfume, and insence. Insence is traditionally used for rituals and religious ceremonies in the Far East. Gaharu is also believed to have tonic and therapeutic properties (Burkill, 1966; Okugawa et al., 1993). Its essential oil is in heavy demand in the perfume industry as evidenced by the recent expansion of the range of uses for Gaharu to include new products such as Gaharu essence, soap and shampoo (chakrabarty et al., 1994).

There are several techniques that allow the extraction of compounds responsible for the aroma of plants. The composition of the aromatic material obtained is strongly dependent on the method of isolation. The main techniques used at industrial scale are cold pressing, hydro distillation, extraction with organic solvents and extraction with compressed CO_2. In Malaysia, the techniques currently practiced in the industry for the extraction of oils are by hydro-distillation and solvent extraction (Nor Azah Mohd. Ali- 2002).Upon hydro distillation of Malaysian

Gaharu oil is obtained in 0.8% yield. These methods were chosen since it is much more suitable for a developing country like Malaysia. The advantages of these techniques are that they are economically viable and safe to operate. The odors of the oil can be described as complex mixture compound.

This essential oil will analyze with different type of fiber via Solid Phase Micro Extraction (SPME) and then injected to Gas Chromatography Mass Spectrometry (GCMS) to identify the chemical compound in Gaharu essential oils. SPME is currently a new and popular technique. It requires minimal accessory equipment low cost and is easy to use. Its major shortcomings are the lack of sensitivity and the limited range of volatiles which can be analyzed. Several chemical compounds such as agarospirol, guaiene, jinkohol and jinkohol 11 have been detected in Malaysian Gaharu oils.

Since Gaharu in the Malaysian market is categorized into several graded, the low quality graded are often distilled to produce essential oils. The essential oil from Gaharu brings high prices due to it rarity and high demand, usually the low grade is around RM30 000 and the superior grades priced up to RM60 000. The higher quality Gaharu wood can be recognized by its darker colour and strong aroma released upon burning its chips or quality incenses.

1.2 Objective

The main objectives of this preliminary study are:

1. To extract the Gaharu oils using hydro distill method.

2. To analyze Gaharu oil with different type of fiber in Solid Phase Microextraction (SPME).

3. To identify the compound of Gaharu oil with different type of column phase via Gas Chromatography Mass Spectrometry (GCMS).

4. To compare chemical compound present in each result from different column phase.

1.3 Scope of Study

The scope of this study is to extract and identified the important chemical constituent in Gaharu oils. We prepare the sample by using the hydro distillation extraction method to extract the essential oil from Gaharu.

The sample will analyze with different type of fiber via Solid Phase Micro Extraction (SPME). Headspace-SPME is identified as a solvent free sample preparation technique in which a fused silica fiber coated with polymeric organic liquid is introduced into the headspace above the sample. The adsorption of the analytes is followed by a thermal desorption process by introducing the SPME fiber into the injection port of a gas chromatography.

In this context, the headspace was considered to be an alternative method to clarify the question about the fragrance of Agarwood oil. The SPME device included a fused silica fiber coating partially cross-linked with 100μm Polydimethylsiloxane (PDMS), 75μm Carboxen/Polydimethylsiloxane (CAR/PDMS) and 65μm Polydimethylsiloxane/divinylbenzene (PDMS/DVB).

The last scope is to understand and know how Gas Chromatography Mass Spectrometry (GCMS) works to identify the compound of Gaharu oil with different type of column phase in GCMS. The column phases that we use are HP-5MS and DB-WAX. The chemical compound that present in the essential oil from Gaharu can be defined.

1.4 Problems Statement

Hydro-distillation is the oldest and most common method of extracting essential oil since it is economically viable and safe. Local institutions like the Forest Research Institute of Malaysia (FRIM) and The Malaysian Timber Industry Board (MTIB) play a major role in the essential oil technology transfer. Even though research were carried out at these institution, lack of documentation and research publication on their part, contributed to this study. Thus, this study want to see whether this method is proven can produce oil and give information on what important step during extraction of Gaharu oils.

Traditionally essential oils have been assessed on the basis of individual requirements. Thus, there is no standard to represent the chemical compounds in Gaharu essential oils in determining their quality. Physically characteristics of essential oils such as density, optical rotation, solubility in solvent, boiling temperature, odor, color and others are easily measured.

Today, the demand for meaningful essential oil standards has increased, because reproducibility of intended effects is essentially determined by a parity of concentration of essential oil component. Once the standard for active compounds in Gaharu essential oil is developed, then the quality of Gaharu oil can be identified. When analyzed their chemical compound via GCMS, that do not provide the full identification of the components and consequently do not give a guarantee of authenticity.

Several of the compounds such as agarospirol, jinkohol-eremol and kusenol have been reported to possibly contribute to the characteristic aroma of Gaharu (Nakanishi et al. 1984; Ishihara et al., 1993). The marker compound isolated will be used for standardizing the Gaharu essential oil contributing to value added products from Malaysian Gaharu and establishing a universal standard for Gaharu in the global market which is presently lacking.

1.5 Rationale and Significant

Hydro distillation method is the best for lab laboratory since its simple, cheap and easy to handle.

A novel method for profiling of essential oils from Aqualaria malaccensis (Gaharu) using newly identified marker compounds will be developed.

Essential oils from Aqualaria malaccensis (Gaharu) that have been identified will acquire added value in the global market.

CHAPTER 2

LITERATURE REVIEW

2.1 Background of Aquilaria Malaccensis (Gaharu)

Aquilaria malaccensis is one of 15 tree species in the Indomalesian genus Aguilaria, family Thymelaeaceae, (Mabberley, 1997). It is large evergreen tree growing over 15-30 m tall and 1.5-2.5 m in diameter, and has white flowers (Chakrabarty et al., 1994). A. malaccensis and other species in the genus Aguilaria sometimes produce resin impregnated heartwood that is fragrant and highly valuable. There are many names for this resinous wood, including agar, agarwood, aloeswood, eaglewood, gaharu and kalamabak, this wood being in high demand for medicine, incense, and perfumes across Asia and the Middle East.

Aquilaria malaccensis is widely distributed in south and south-east Asia. There are differing accounts of the countries in which it occurs. According to Oldfield et al. (1998), A. malaccensis is found in 10 countries such as Bangladesh, Bhutan, India, Indonesia, Iran, Malaysia, Myanmar, Philippines, Singapore and Thailand.

Aquilaria species have adapted to live in various habitats, including those that are rocky, sandy or calcareous, well drained slopes and ridges and land near swamps. They typically grow between altitudes of 0-850 m, in locations with average daily temperatures of 20-22^{o}C (Afifi, 1995; Keller and sidiyasa, 1994; Wiriadinata, 1995).

Formation of Aquilaria occurs in the trunk and roots of trees that have been infected by a parasite ascomycetous mould, Phaeoacremonium parasitica, a dematiaceous (dark-walled) fungus. As a response, the tree produces a resin high in volatile organic compounds that aids in suppressing or retarding the fungal growth. While the unaffected wood of the tree is relatively light in colour, the resin dramatically increases the mass and density of the affected wood, changing its colour from pale beige to dark brown or black. In natural forest only about 7% of the trees are infected by the fungus. A common method in artificial forestry is to inoculate all the trees with the fungus. (http://www.organicessentialoils.in/AgarwoodOil.html).

High quality resin comes from a tree's natural immune response to a fungal attack. It is commonly known as agarwood. An inferior resin is created using forced methods where aquilaria trees are deliberately wounded, leaving them more susceptible to a fungal attack.

Some low cost agarwood oil is also developed through producers names Oud and high used in cosmetic preparations and other places. This quality is quite infamous and the oils are developed in particular carrier oil. Agar is the pathological product of a fungal disease contracted by the tree chiefly through wounds on the trunk. Since agar is located deep within the trunk, its detection is not easy. Generally such trees are distinguished by poor crown development, the presence of swellings or depressions on the bole. Depending upon the extent of the resin accumulation the heartwood is graded into four categories:

- Grade 1 Black/True Agar: mainly exported to Arabia as incense
- Grade 2 Bantang: mainly exported to Arabia as incense
- Grade 3 Bhuta or Phuta: sometimes extracted for a superior oil
- Grade 4 Dhum: used for oil

In true of black agar, the impregnation of the resin is intense and the wood resembles black stone. It is heavy to the extent that it sinks in water, and bears the

highest content but it is difficult to distill. True agar is mainly exported to the Middle East countries where it is used as incense. Bantang is brown in color without any black tone. Bhuta is also brown in color but interspersed with 50 per cent or more of yellow-colored wood. These two grades are also usually used in incense. Dhum is the lowest grade which is mostly yellow with scattered streaks of brown or black resin. It is chiefly distilled for the oil. Sometimes oil is also extracted from Bhuta and this oil is reported to be superior to that from Dhum.
(http://members.aol.com/ratrani/agarwood.html).

Figure 2.1: Pieces of *Aquilaria* wood lacking the dense and dark resinous agarwood caused by infection

Figure 2.2: Colour is one criterion in assessing the value of a gaharu. The darker the gaharu is, the higher value it fetches. The black and shiny resin on the top right (circled) is of Grade A.

(Source: http://www.forestry.gov.my/pdf/NST220308.pdf)

2.2 Aquilaria Malaccensis in Malaysia

A.malaccensis is distributed throughout Peninsular Malaysia, except for the States of Kedah and Perlis (Barden et al., 2000), but although the species has good geographical coverage, its occurrence in rather rare, with trees often locally scattered. La Frankie (1994) studied the population dynamics of A.malaccensis in Pasoh Forest Reserve and suggested a typical lowland Malaysia forest density of 2.5/ha and found that the growth rate varied between 0-1.95 cm/year.

Malaysia has a long history in the trade in A.malaccensis, which has long been collected by the indigenous peoples of the interior of Peninsular Malaysia, Sabah and Sarawak to supplement their income. In Peninsular Malaysia, the A.malaccensis products in domestic trade are woodchips and powder or sawdust (Chua, 2003). Some use has been recorded locally for medicinal purposes, but it appears that the majority of A.malaccensis harvested is exported (Barden et al.2000). The wood is also used for making small boxes in Sabah (Sabah Forest Department, 2003).

2.3 Uses of Gaharu

Both Gaharu wood and oil are highly prized for the scent produced. The unique Gaharu scent is released on burning the resinous wood. Many uses are recorded for Gaharu.

2.3.1 Incense

One of the traditional uses of Gaharu is for the production of incense, and is by far the most prized of all incenses. Incense made from high quality Gaharu is very expensive. Prices vary with the graded used. It is used in important religious ceremonies, rituals and meditation.

In the Arabian Country's People are used Agarwoods as an incense and burn it on coal. Both agarwood oil and incense are used for their fragrant properties, notably in the Middle East. Buddhist, Hindus and Muslims use Agarwood incense in religious ceremonies, while a revival of the "Koh doh" incense ceremony in Japan has rekindled interest in agarwood in that country.(http://essentialoilscompany.com).

2.3.2 Medicinal Uses

In Malaysia, Gaharu is used in various folk remedies for the treatment of weakness, stomach pains, after pregnancy, fever, chest pains, body pains, rheumatism, women diseases and dropsy (Chang, et al. 2002). Gaharu also used in curry flavor. A decoction of the wood used for abdominal pain, asthma, cancer, colic chest, congestion, diarrhea, hiccups, nausea, nerves and also regurgitation (Kim et al. 1997).

Gaharu have been recorded in traditional medical systems including Chinese (TCM), Tibetan, Ayurvedic (Indian) and Unani (Greek derived Islamic). External and Internal preparations have been used citing a variety of *Aquilaria* species. (http://www.equitech.biz/equitech_Silviculture)

- **TCM**

  Formulations in general seem to relieve spasms and other forms of stagnant or stuck energy particularly in the digestive (stomach, kidneys, liver, and bowel) and respiratory systems.

- **Ayurveda**

  In the Indian Ayurvedic healing system, the burning of agarwoods has a warming and centering effect on the chakras and promotes a deep meditational state. Agarwood heartwood is used in various Ayurvedic formulas including Chyavanprasha, Arimedadi Taila and Mahanarin Taila. Its uses have been described as a cardiac tonic, carminative & refrigerant.

- **Unani**

  It is used as a stimulant, stomachic, laxative (purgative in large doses) and as an aphrodisiac. It is also used in the Ayurvedic system against skin diseases and powdered heartwood is given for treatment of diahorrea, dysentery, vomiting and anorexia. Agarwood oil, mixed with essential oil from Piper betel is used against bronchial asthma. It is also reported as being used by the traditional vaidyas as a contraceptive and the leaves boiled in oil used to remove fish bones stuck in the throat.

- **Tibetan Medicine & Ethinic Psychiatry.**

  Oleoresin, wood and oil are used in Tibetan medicine and incense, especially prized is “black aloeswood”, (*Aquilaria agallocha*) which Clifford (1984) describes as being relied on by contemporary Tibetan doctors for treatment of a whole range of nervous and emotional disorders. Clifford further describes black aloeswood as the most commonly used minor tranquilliser.

2.3.3 Aromatherapy

Gaharu perfumes comprise Gaharu oil mixed with a carrier such as sandalwood oil. 'Attar oil,' for example, is water-based perfume containing Gaharu oil that is normally used by Muslims to lace prayer clothes. Gaharu essences have also recently been used to fragrance soaps and shampoos.

Miller and Miller (1995) in their book Ayerveda Aromatherapy *is* the energetic warming, balancing effects of oud (oil of *A. agallocha),* and its’ energy purifying and balancing, relaxant, rejeuvinative, transformative, clairvoyant and transcending actions. (http://www.equitech.biz/equitech_Silviculture)

2.4 Structure cell of Gaharu

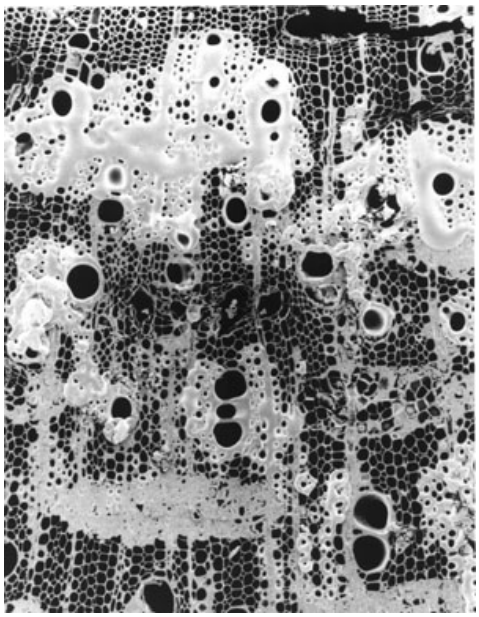

Figure 2.3: Cross section of Gaharu Cell

(Source: http://forestpathology.cfans.umn.edu/agarwoodadd.htm)

A cross section of an experimental tree with Gaharu observed by scanning electron microscopy showing copious amounts of resin formed in the wood cells. *Aquilaria* has an unusual anatomy and specialized cells within the xylem produce the resin. It is in species which have included phloem in the secondary cambium.

2.5 Essential Oil

Essential oils can be any products from herbal plans & tree who find via Hydro distill plant, CO_2 Plant (Supercritical Carbon Dioxide), Solvent Extraction, Cold-Pressed etc. Its can be any Oil, Concretes, Natural Aroma Compounds, Perfumes from the Leaves, Flowers, Bark, Roots, Wood, Seeds or Peel of Herbal Plans & Tree.

Essential oils are pure, natural and extremely concentrated substances derived from the flowers, leaves, stems, seeds, peels or barks of difference plants. They are